A Combined Chemical–Enzymatic Synthesis of 3-Deoxy-D-*arabino*-heptulosonic Acid 7-Phosphate[1]

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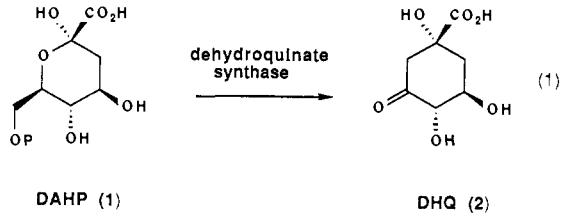
Abstract: 3-Deoxy-D-*arabino*-heptulosonic acid 7-phosphate (DAHP) has been synthesized from *N*-acetyl-D/L-aspartate β-semialdehyde and dihydroxyacetone phosphate in four steps with an overall yield of 13%. The key step generates the required threo stereochemistry by using rabbit muscle aldolase (E.C. 4.1.2.13) as catalyst.

3-Deoxy-D-*arabino*-heptulosonic acid 7-phosphate (DAHP, **1**) is an important intermediate in the shikimic acid pathway, standing at the crossover point from the sugar phosphates to the cyclitols that eventually are transformed into the aromatic amino acids.[3-5] This cascade of transformations is initiated by the conversion of DAHP (**1**) to dehydroquinate (DHQ, **2**), catalyzed by dehydroquinate synthase (DHQ synthase) (eq 1).



(1)

The inhibition of this reaction, and the resulting disruption of aromatic amino acid biosynthesis in plants, provides the basis for the activity of certain herbicides.[6] The development of new plant growth regulators of this type would be facilitated by a convenient synthetic route to DAHP; such a route might also allow for the preparation of structural analogues of DAHP.

Most of the previous chemical syntheses of DAHP[7-11] have employed 2-deoxy-D-glucose (a relatively costly carbohydrate at $17/g) as the starting material. The most efficient of these syntheses[10] requires eight steps and proceeds in 6% overall yield. Recently, Frost and co-workers[12] have used an enzymatic system to produce DAHP from D-fructose. Despite the efficiency of this methodology (85% conversion of D-fructose to DAHP) the cost and sensitivity of the enzymes involved are such that this method is not a practical one for the production of DAHP. This paper explores a new approach to the synthesis of DAHP that uses rabbit muscle aldolase[13-15] (E.C. 4.1.2.13) to catalyze formation of the C4–C5 bond. This aldol condensation generates these two chiral centers disasteriospecifically (eq 2). The successful execution of this approach is summarized in Scheme I.

Scheme I. Synthesis of DAHP, **1**

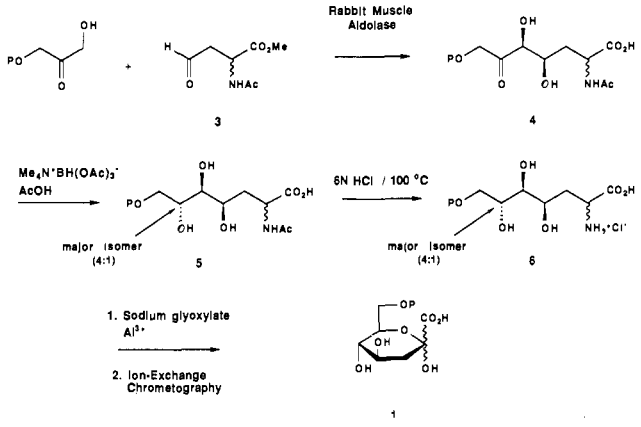
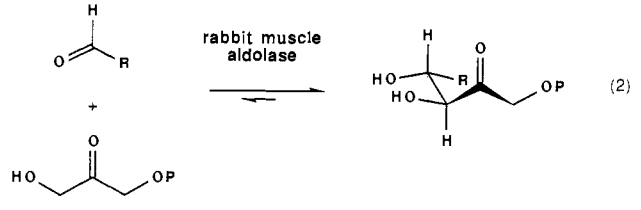


Table I. Diastereoselectivity of Reduction of **4**

reagent	solvent	temp (°C)	time (h)	anti:syn[a,b]
$Na^+BH_4^-$	H_2O	25	1	45:55
$Na^+BH_4^-/CeCl_3$	H_2O	25	1	50:50
$Na^+BH(OAc)_3^-$	AcOH	25	2	75:25
$Me_4N^+BH(OAc)_3^-$	AcOH	25	2	80:20
$Me_4N^+BH(OAc)_3^-$	AcOH[c]	0	24	78:22

[a] Diastereomeric ratios were determined by ^1H NMR spectroscopy (500 MHz). Integration of the *N*-acetyl signals due to the 2*R* isomer are reported although the 2*S* isomer gave essentially the same ratios. [b] The assignment of anti and syn was made as follows: the product from entry 4 was converted to the diastereomeric mixture of amino acids **6** (see Scheme I). Treatment with sodium glyoxylate generated a mixture of DAHP (**1**) together with its C-6 epimer (ratio 80:20 respectively) thus confirming the predominantly anti reduction. [c] This experiment was carried out on the tetramethylammonium salt of **4**. Acetonitrile was used as a cosolvent to prevent freezing of the solution.



(2)

Results and Discussion

Treatment of *N*-acetyl-D/L-allylglycine[16] with diazomethane afforded the corresponding methyl ester in quantitative yield. Ozonolysis, followed by reductive workup with dimethyl sulfide, gave the aldehyde **3** in 75% yield after chromatography. In

(1) Supported by NIH Grant GM 30367.
(2) N.J.T. was a Royal Society Research Fellow, 1985–1987.
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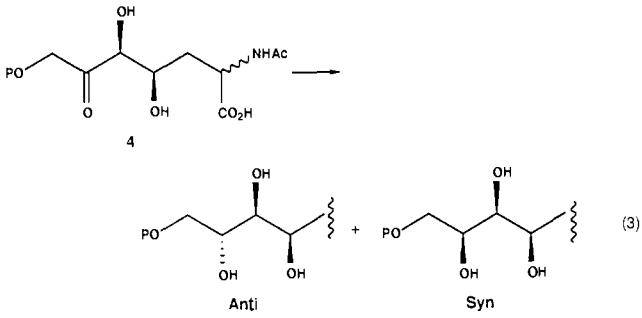
practice, the crude aldehyde was used in the enzymatic reactions since the major byproduct of the ozonolysis (dimethyl sulfoxide) did not influence the activity of aldolase at concentrations below approximately 20%.[17]

The reaction of aldehyde **3**[18] with dihydroxyacetone phosphate[19] in the presence of aldolase was monitored by ^{1}H NMR spectroscopy. Conversion reached a maximum of 40%. Ion-exchange chromatography of the product mixture gave **4** in 37% yield. In addition to the expected carbon–carbon bond formation, hydrolysis of the methyl ester also occurred.[20] Both ^{31}P and ^{1}H NMR spectroscopy showed the product to be a mixture of diastereomers, epimeric at C-2 (2R:2S = 1.5:1.0).[21,22]

The sodium salt of **4** was reduced by using a variety of reagents and conditions (eq 3; Table I, Experimental Section). Since the



anti disastereomer was required, we focused our attention on the use of the triacetoxyborohydrides.[23] Although the diastereoselectivities were lower than had previously been observed[23,24] (possibly due to the influence of the neighboring phosphate group), both sodium and tetramethylammonium triacetoxyborohydride gave the desired anti configuration as the dominant isomer (anti:syn = 4:1).[25] No attempt was made to separate these diastereomers.

Hydrolysis of the crude reaction product containing **5** was carried out at 100 °C in 6 N HCl for 90 min. Under these conditions removal of the *N*-acetyl group occurred with only minimal cleavage of the phosphate (as evidenced by TLC). Purification on ion-exchange resin gave a diastereomeric mixture of amino acids **6**.

The final transformation of **6** to the corresponding ketoacid was troublesome. We had hoped to use the commercially available D- and L-transaminases to accomplish this transformation, but the mixture of amino acids **6** showed no reaction with either enzyme. We then turned to some conventional chemical methods, namely trifluoroacetic anhydride[26] and 3,5-di-*tert*-butyl-*o*-benzoquinone,[27] but again were only able to recover either starting material or unidentified products from these reactions. The final step was eventually accomplished by a transamination reaction with sodium glyoxylate.[28] Addition of only 1 equiv of sodium glyoxylate gave rise to an equilibrium mixture of starting material and two diastereomeric products. Five equivalents were necessary to cause complete conversion to products. Separation of the two diastereomers by ion-exchange chromatography yielded pure DAHP **1**. ^{1}H NMR indicated that the sample was ~90% pure. Repurification of an aliquot (~10 mg) of the synthetic DAHP by chromatography on DE-52 increased the purity to ~95% as judged by ^{1}H NMR.

The synthetic DAHP (from DE-52 column) was a substrate for DHQ synthase. The value of the initial rate obtained (0.123 AU/min) was, however, significantly below that recorded in a parallel experiment with authentic DAHP (0.334 AU/min).[29] This low value was established kinetically to be due to the presence of a competitive inhibitor in the synthetic DAHP. Although we did not determine the structure of this compound, its influence on the rate of reaction of pure DAHP demonstrated that it was a potent inhibitor. Determination of its structure might suggest leads to new inhibitors of DHQ synthase.

In summary this route to DAHP has four attractive features. First, the protection and deprotection steps are minimized, and all reactions are carried out in aqueous media. Second, the introduction of the phosphate group, often a step proceeding in low yield, is accomplished simultaneously with formation of the carbon–carbon bond. Third, the preparation of isotopically labeled DAHP **1** can be readily accomplished by using this method with appropriately labeled aldehyde **3** or dihydroxyacetone phosphate. Fourth, the high tolerance of rabbit muscle aldolase for the aldehyde component suggests that this route offers a potentially useful method for synthesizing analogues of DAHP, with the possibility of structural modification at any centers other than C-4 and C-5 (the centers formed in the aldolase-catalyzed reaction).

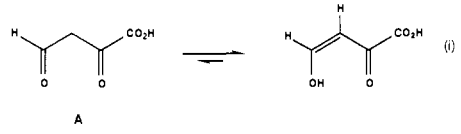
The synthesis of DAHP by the sequence of reactions in Scheme I demonstrates the use of rabbit muscle aldolase to synthesize amino sugars **4**, **5**, and **6** from an aldehyde derived from an amino acid and suggests the use of this enzyme for the preparation of other amino sugars.

Experimental Section

General Methods. TLC plates were visualized by immersion in anisaldehyde stain (by volume: 93% ethanol, 3.5% sulfuric acid, 1% glacial acetic acid, and 2.5% anisaldehyde) followed by heating. AG 1-X8 and AG 50W-X8 were purchased from Bio-Rad (100–200 mesh). Aldolase (rabbit muscle, E.C. 4.1.2.13), was obtained in lyophilized form from Sigma Chemical Co. Dihydroxyacetone phosphate was prepared according to the method of Wong et al.[13] Satisfactory analyses were not obtained for compounds **4**, **5**, and **6** owing to the difficulty experienced in preparing stable derivatives. The purity of each was judged to be

(17) Bischofberger, N.; Whitesides, G. M. Unpublished results.

(18) Although our initial plan had been to use the aldehyde **A** (eq i) as the precursor, we observed no reaction of **A** with dihydroxyacetone phosphate in the presence of aldolase. This unreactivity is most likely due to the high enol content of **A** (α,β-Unsaturated carbonyl compounds inactivate aldolase. Effenberger, F.; Straub, A. *Tetrahedron Lett.* **1987**, *28*, 1641).



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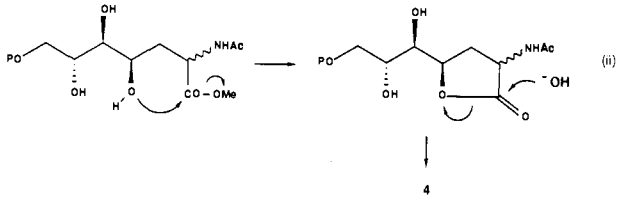
(20) It is likely that hydrolysis of the methyl ester occurred after carbon–carbon bond formation since a solution of the aldehyde **3** in D_2O showed only slight hydrolysis (less than 5%) after 72 h at 25 °C. The relative lability of the methyl ester group is probably due to intramolecular nucleophilic assistance from the C(4) hydroxyl (eq ii). Further support for this rationale comes from the ease with which these compounds were found to undergo lactonization under acidic conditions.



(21) The assignment of the diastereomers was made by repeating the aldolase reaction with the aldehyde derived from L-allylglycine, thereby generating the 2S isomer of **4**.

(22) We have previously observed kinetic selectivity by aldolase in similar reactions: Bednarski, M. D.; Lees, W.; Kim, M. J.; Whitesides, G. M. Unpublished results.

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(25) Examination of the ^{1}H NMR spectrum (D_2O, pH 1.5) of the crude product containing **5** indicated the presence of a new set of resonances in addition to those signals arising from **5** (ratio of unknown/**5** = 10:1). Adjustment of the pH to 7.5 caused the disappearance of these signals, and the spectrum now consisted entirely of **5**. We tentatively assign these signals to a diastereomeric mixture of lactones.

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(29) We thank our colleague Professor J. R. Knowles, Harvard University, for the gift of an authentic sample of DAHP.

~95% by ^{1}H NMR and ^{13}C NMR.

N-Acetyl-D/L-allylglycine Methyl Ester. N-Acetyl-D/L-allylglycine[7] (8.0 g, 50 mmol) was dissolved in ethyl acetate (100 mL) and cooled to 0 °C. Diazomethane (a solution in ether) was added dropwise to the stirred solution until the yellow color persisted for 5 min. The excess diazomethane was destroyed with glacial acetic acid, and the solvent was removed in vacuo to give the methyl ester (8.7 g, 100%): IR (film) 3270 (s), 3070 (m), 2940 (m), 1740 (s), 1655 (s), 1530 (s), 1435 (m), 1365 (m), 1210 (s), 1145 (m), 990 (m), 915 (m) cm^{-1}; ^{1}H NMR (250 MHz, $CDCl_3$) δ 1.98 (s, 3 H), 2.39–2.62 (m, 2 H), 3.70 (s, 3 H), 4.59–4.67 (m, 1 H), 5.02–5.14 (m, 2 H), 5.58–5.74 (m, 1 H), 6.18–6.32 (d, J = 5 Hz, 1 H); ^{13}C NMR (125.7 MHz, $CDCl_3$) δ 22.63, 36.12, 51.52, 52.03, 118.69, 132.12, 169.89, 172.13; MS (CI, isobutane), 172 (MH$^+$, 100), 140 (7), 130 (8), 112 (4): exact mass calcd for $C_8H_{14}NO_3^+$ 172.09736, found 172.09742.

Methyl N-Acetyl-D/L-aspartate β-Semialdehyde (3). The protected amino acid (8.7 g, 50 mmol) was dissolved in CH_2Cl_2 (40 mL) and CH_3OH (10 mL) and cooled to −78 °C. Ozone was bubbled through until the solution became pale blue. Nitrogen was passed through to remove the excess ozone. Dimethyl sulfide (3 mL) was added, and the solution was stirred overnight. The solvent was removed in vacuo, and the crude product was chromatographed on silica (ethyl acetate) to give the aldehyde **3** (400 mg, 75%): IR (film) 3280 (s), 2950 (m), 1740 (s), 1650 (s), 1530 (s), 1435 (m), 1370 (m), 1220 (s), 1140 (m), 1040 (m), 725 (m) cm^{-1}; ^{1}H NMR (500 MHz, $CDCl_3$) δ 2.02 (s, 3 H), 3.06–3.19 (m, 2 H), 3.76 (s, 3 H), 4.83–4.87 (m, 1 H), 6.44–6.52 (d, J = 7 Hz, 1 H), 9.72 (s, 1 H); ^{13}C NMR (125.7 MHz, $CDCl_3$) δ 22.50, 45.17, 46.98, 52.48, 170.18, 171.05, 199.34; MS (CI, isobutane), 174 (MH$^+$, 100), 132 (13), 114 (5): exact mass calcd for $C_7H_{12}NO_4^+$ 174.07663, found 174.07655.

(2*RS*, 4*R*, 5*S*)-2-Acetamido-6-oxo-4,5,7-trihydroxyheptanoic Acid, 7-(Dihydrogen phosphate) (4). A solution of dihydroxyacetone phosphate[13] (19.5 mmol) in distilled water (250 mL) was adjusted to pH 6.8 with 2 N NaOH. To this solution was added the crude product containing the aldehyde **3** (3.00 g, 75% pure, 13.0 mmol), the pH was readjusted to 6.8, and the solution was then purged with N_2 for 30 min. Aldolase (45 mg, 500 U) was added, and the reaction mixture was shaken at 125 rpm and 27 °C. After 22 h the pH was readjusted to 6.8, and additional aldolase (16 mg, 200 U) was added. The degree of conversion was estimated by using the following protocol: an aliquot (1 mL) of the reaction was removed, lyophilized, and resuspended in D_2O (0.60 mL). An accurately measured quantity (0.05 mL) of a solution of 0.75% sodium 3-(trimethylsilyl)propionate-2,2,3,3,-d_4 (TSP-d_4) was added, and the ^{1}H NMR spectrum was measured at 500 MHz. Integration of the resonances of the product against TSP-d_4 gave the following values for the extent of conversion (error 5%): 4.5 h (10%), 22 h (20%), 28.5 h (32%), 47 h (37%), 71 h (40%). The reaction was terminated after 71 h, diluted to 500 mL with distilled water, and applied to a column of AG 1-X8 resin (HCO_3^- form, 75 mL), followed by elution with 250 mL each of the following concentrations of triethylammonium bicarbonate: 150, 200, 300, 350 mM. Fractions (volume = 18 mL, total no. of fractions = 70) were collected and examined by TLC. Fractions 23–50 contained the desired product and were pooled and lyophilized; excess buffer was removed by the addition of water (15 mL) to the residue and reevaporation (3 times). The white crystalline solid thus obtained was redissolved in water (150 mL) and passed down a column of AG 50W-X8 resin (Na$^+$ form, 50 mL). A further 100 mL of water was added, and the combined eluant was concentrated in vacuo to give the trisodium salt of **4** (1.88 g, 37%): $[\alpha]_D$ +8.8° (c 1.00, H_2O); IR (Nujol) 3250 (s), 1730 (m), 1610 (s), 1070 (s), 970 (m), 920 (m) cm^{-1}; ^{1}H NMR (500 MHz, D_2O, pH 6.5) δ 1.79–2.22 (complex m, 2 H), 2.05 (s, 1.2 H), 2.06 (s, 1.8 H), 4.14–4.17 (m, 0.67 H), 4.19–4.24 (m, 0.67 H), 4.31 (dd, J = 3.5, 11 Hz, 0.67 H), 4.42 (d, J = 2 Hz, 0.67 H), 4.45 (d, J = 2 Hz, 0.33 H), 4.47–4.86 (m, 2 H); ^{13}C NMR (125.7 MHz, D_2O, pH 6.5) δ 22.70, 35.64, 35.82, 52.84, 53.44, 69.08, 69.23 (J_{POC} = 7 Hz), 70.03, 77.62, 78.73, 174.15, 174.63, 179.18, 179.81; ^{31}P NMR (121.47 MHz, D_2O, pH 6.5) δ 0.79, 0.83 (ratio 1.0:1.5); m/z (positive argon, fast atom bombardment) 396 (MH$^+$).

General Procedure for the Reduction of 4. (A). In Water. To a solution of the trisodium salt of **4** (30 mg, 0.070 mmol), dissolved in water (3 mL), was added the appropriate borohydride reagent. After stirring for 1 h, the reaction was quenched by lowering the pH to 5 with 0.1 M HCl. The pH was readjusted to 7 with 0.1 M NaOH and then applied to a column of AG 1-X8 resin (HCO_3^- form, 5 mL). After washing the column with triethylammonium bicarbonate (100 mM, 100 mL), the product was eluted with triethylammonium bicarbonate (300 mM, 100 mL). Lyophilization of the 300 mM fraction gave the triethylammonium salt of **5**. **(B). In Acetic Acid.** To a solution of the trisodium salt of **4** (30 mg, 0.070 mmol), dissolved in anhydrous glacial acetic acid (3 mL), was added the triacetoxyborohydride (3 equiv), and the mixture was stirred for 2–24 h. The solvent was removed in vacuo (azeotropically by the addition of heptane, 3 × 5 mL), the residue was redissolved in water, and the pH was adjusted to 7 with 0.1 M NaOH. Purification on AG 1X-8 resin was carried out as described in **A**.

Results of reductions designed to test the stereoselectivity of several reduction procedures are summarized in Table I.

(2*RS*,4*R*,5*S*,6*RS*)-2-Acetamido-4,5,6,7-tetrahydroxyheptanoic Acid, 7-(Dihydrogen phosphate) (5). The trisodium salt of the aldol product **4** (800 mg, 2.03 mmol) was dissolved in anhydrous glacial acetic acid (10 mL). To this solution was added tetramethylammonium triacetoxyborohydride (1.58 g, 6.0 mmol, 3 equiv) in anhydrous glacial acetic acid (8 mL), and the mixture was stirred at room temperature for 2 h. Excess acetic acid was removed in vacuo (by azeotropic distillation with heptane, 3 × 5 mL), and the resulting solid was taken up in water (50 mL). Sufficient AG 50W-X8 resin (H$^+$ form) was then added to adjust the pH to 1.5, and the resin was removed by filtration. To the filtrate was added methanol (50 mL). The solvent was removed in vacuo at a water bath temperature of 40 °C, and the resulting gummy solid redissolved in water (25 mL) and methanol (25 mL) followed by evaporation to dryness. Drying under high vacuum for 3 h yielded the hydrogen form of **5** as a yellow solid (660 mg, 98%). For characterization, a sample (100 mg) was removed and dissolved in water (5 mL), and the pH was readjusted to 7 with dilute NaOH and followed by dilution to 20 mL with water (50 mL). The solution was applied to a column of AG 1-X8 resin (HCO_3^- form, 10 mL) and eluted with the following concentrations (volume) of triethylammonium bicarbonate: 100 nM (100 mL) and 300 mM (100 mL). Concentration of the latter fraction, evaporation with 2-propanol (3 times, 10 mL), gave the triethylammonium salt of **5**. This white solid was redissolved in water (20 mL) and applied to a column of AG 50 W-X8 resin (Na$^+$ form, 10 mL), eluted with water (200 mL), and lyophilized to give **5** as the trisodium salt (107 mg, 90%): IR (Nujol) 3250 (s), 1590 (s), 1300 (w), 1070 (s), 965 (m), 920 (m) cm^{-1}; ^{1}H NMR (500 MHz, D_2O, pH 6.0) δ 1.71–2.22 (complex m, 2 H), 2.036 (s, 0.96 H), 2.039 (s, 0.24 H), 2.054 (s, 0.36 H), 2.055 (s, 1.44 H), 3.48–4.08, 4.20–4.25, 4.32–4.38 (complex m, 6 H); ^{13}C NMR (125.7 MHz, D_2O, pH 6.0) δ 22.76, 35.94, 36.08, 36.46, 52.95, 53.19, 53.74, 53.81, 66.50, 66.75, 66.93, 68.05, 69.09, 70.02, 71.02, 71.72, 72.06, 73.49, 74.20, 171.84, 174.23, 174.64, 179.69, 179.79, 180.22, 180.30; ^{31}P NMR (121.47 MHz, D_2O, pH 6.5) δ 2.25, 2.69; m/z (positive argon, fast atom bombardment) 398 (MH$^+$).

(2*RS*,4*R*,5*S*,6*RS*)-2-Amino-4,5,6,7-tetrahydroxyheptanoic Acid, 7-(Dihydrogen phosphate) (6). The crude product containing **5** (600 mg, 1.8 mmol) was dissolved in 6 N HCl (30 mL) and heated at 100 °C for 60 min (TLC indicated complete removal of starting material). Removal of water gave a dark yellow solid that was redissolved in water (100 mL). The pH was adjusted to 7.0 with 1 M NaOH, and the solution was applied to a column of AG 1-X8 resin (HCO_3^- form, 30 mL). The column was sequentially eluted with the following concentrations (volume) of triethylammonium bicarbonate: 100 mM (200 mL), 150 mM (200 mL), 200 mM (200 mL), and 250 mM (200 mL). The product was located with ninhydrin in both the 150- and 200-mM fractions. These were concentrated, redissolved in water (50 mL), and passed down a column of AG 50W X-8 resin (H$^+$ form, 30 mL), followed by elution with water (50 mL). The combined fractions were lyophilized and redissolved in water (50 mL), the pH was adjusted to 7.0 with 1 M NaOH, and the fractions were lyophilized to give the disodium salt of **6** (390 mg, 65%): IR (Nujol) 3200 (s), 1625 (s), 1530 (w), 1070 (s), 965 (m), 720 (w) cm^{-1}; ^{1}H NMR (500 MHz, D_2O, pH 7.0) δ 1.96–2.33 (complex m, 2 H), 3.19–4.12 (complex m, 6 H); ^{13}C NMR (125.7 MHz, D_2O, pH 7.0) δ 34.21, 34.37, 34.89, 53.26, 53.49, 54.37, 65.85, 67.82, 68.99, 69.30, 71.17, 71.86, 72.91, 73.29, 74.41, 175.42; ^{31}P NMR (121.47 MHz, D_2O, pH 7.0) δ 4.06, 4.50, 4.54, 4.80; m/z (positive argon, fast atom bombardment) 334 (MH$^+$).

3-Deoxy-D-*arabino*-heptulosonic Acid 7-Phosphate (1). To a solution of the disodium salt of **6** (210 mg, 0.63 mmol), dissolved in water (30 mL), were added sodium glyoxylate (350 mg, 3.15 mmol, 5 equiv) and potassium aluminum sulfate (30 mg, 0.063 mmol, 10 mol %). The pH was adjusted to 5 with 1 M HCl solution, and the mixture was heated at 100 °C. After 2 h, TLC indicated the complete disappearance of starting material. The crude solution containing DAHP was allowed to cool to room temperature, neutralized to pH 7 with 1 M NaOH, and applied to a column of AG 1-X8 resin (HCO_3^- form, 20 mL). The column was eluted first with triethylammonium bicarbonate (100 mM, 200 mL) followed by a linear gradient of triethylammonium bicarbonate (300 mL of 150 mM solution to 300 mL of 250 mM solution). Fractions containing DAHP (located by TLC against an authentic sample) were pooled and lyophilized. Excess buffer was removed by the addition of water and reevaporation (10 mL, 3 times). The resulting white solid was redissolved in water (20 mL) and passed down a column of AG 50W-X8 resin (H$^+$ form, 20 mL). The eluant was brought to pH 5.0 by the careful addition of 0.1 M lithium hydroxide solution and then lyophilized

to give the dilithium salt of **1** (104 mg, 55%) (~90% pure by ^{1}H NMR): $[\alpha]_D$ +19.8° (*c* 0.50, H_2O), authentic[30] +18.6°; ^{1}H NMR (500 MHz, D_2O, pH 5.0) δ 1.80 (dd, *J* = 12, 13 Hz, 1 H), 2.19 (dd, *J* = 5, 3 Hz, 1 H), 3.53 (overlapping dd, *J* = 10 Hz), 3.82-3.87 (m, 1 H), 3.92-3.97 (m, 1 H), 4.07-4.17 (m, 1 H); ^{13}C NMR (125.7 MHz, D_2O, pH 5.0) δ 39.87, 64.91, 69.38, 71.14, 73.52 (d, J_{POC} = 7 Hz), 97.12, 177.17; ^{31}P NMR (121.47 MHz, D_2O, pH 5.0) δ 1.09.

A sample of **1** (5 mg) was applied to a column of Sephadex DEAE A-25 ion-exchange resin (HCO_3^- form, 10 mL) and eluted with a linear gradient to triethylammonium bicarbonate (150 mL of 100 mM to 150 mL of 350 mM). The DAHP containing fractions were pooled and lyophilized to give a white solid that was redissolved in water (10 mL) and passed down a column of AG 50W-X8 resin (H^+ form, 10 mL). Adjustment of the eluant to pH 5.0 with 0.1 M lithium hydroxide, followed by lyophilization, gave the dilithium salt of **1** (3 mg) (^{1}H NMR indicated ~95% purity). This sample was used for the assay with dehydroquinate synthase.

Assay of Synthetic DAHP (1) with Dehydroquinate Synthase. The assay procedure for DAHP used a coupled enzyme system of dehydroquinate synthase and dehydroquinase with subsequent monitoring of dehydroshikimate production. Assay solutions (1.00 mL) containing 50 mM MOPS buffer, pH 7.50, cobalt sulfate (50 μM), NAD^+ (15 μM), DAHP (500 μM), and 2 units of dehydroquinase were incubated at 20 °C in quartz cuvettes. The reaction was initiated by the addition of 800 milliunits of DHQ synthase, and the production of dehydroshikimate was monitored at 234 nm. Initial rates obtained from the first ~20 s after mixing were as follows: synthetic DAHP, 0.123 AU/min; authentic DAHP, 0.334 AU/min.

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Rabbit Muscle Aldolase as a Catalyst in Organic Synthesis[1]

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Abstract: D-Fructose-1,6-bisphosphate aldolase from rabbit muscle (RAMA, EC 4.1.2.13) is a synthetically useful catalyst for the stereoselective aldol condensation between dihydroxyacetone phosphate (DHAP, **1**) and aldehydes having a range of structures. This paper demonstrates that more than 50 aldehydes [in addition to its natural substrate, D-glyceraldehyde 3-phosphate, G-3-P (**2**)] are substrates for RAMA. Unhindered aliphatic, α-heteroatom-substituted, and differentially protected alkoxy and amino aldehydes are substitutes; sterically hindered aliphatic and α,β-unsaturated aldehydes are not. Aromatic aldehydes are either poor as substrates or they do not react. Phosphorylated aldehydes react more rapidly than their unphosphorylated counterparts, and aldehydes substituted in the α position with an electronegative group react more rapidly than unsubstituted aliphatic aldehydes. Permissible variations in the structure of the DHAP moiety are much more restricted; only two—1,3-dihydroxy-2-butanone 3-phosphate (**68**) and 1,4-dihydroxy-3-butanone-1-phosphonate (**69**)—of 11 DHAP analogues tested were substrates for RAMA. RAMA is stable under the reaction conditions used in synthetic applications: it withstands organic cosolvents (up to 20% DMSO or EtOH); it can be used in immobilized form, in soluble form, or enclosed within a membrane; it is also air-stable. Syntheses of 5,6-dideoxy-D-*threo*-2-hexulose 1-phosphate (**82**), 5-*O*-methyl-D-fructose (**85**), D-xylulose (**88**), and peracetylated D-*glycero*-D-*altro*-2-octulose (**91**) on scales of 4 mmol-1 mol demonstrate practical application of this catalytic synthetic methodology. In a limited study of kinetic diastereoselectivity, RAMA exhibits useful selectivity in two cases: with (±)-glyceraldehyde 3-phosphate [**2**; 20:1 ratio, the major isomer being D-fructose 1,6-bisphosphate (**3**)] and with (±)-2-(benzyloxy)propanal [**19**; 3:1 ratio, the major isomer being (*S*,*S*,*R*)-5-(benzyloxy)-3,4-dihydroxy-1-(phosphonooxy)-hexan-2-one (**91**)]. When (±)-3-hydroxybutanal (**100**) reacts with DHAP, high thermodynamic diastereoselectivity was observed: the ratio of the two stereoisomers (**103** to **104**) is 97:3 and the major isomer is 5,7-dideoxy-L-*gluco*-2-heptulopyranose 1-phosphate (**103**).

The development of methods for stereoselective formation of carbon-carbon bonds using the aldol reaction is a current focus of effort in organic synthesis.[7-12] Many successful strategies using chiral auxiliaries have been reported.[13-21] In principle, however, a catalytic asymmetric aldol reaction requiring minimal effort to prepare the catalyst would be preferable to methods requiring a stoichiometric equivalent of a chirotopic auxiliary. Previous reports[22] describe three nonbiological catalysts—Zn(II) complexes

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